Heliyon 8 (2022) e09038

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Bacterial microbiota of the contact lens surface and associated care behaviours

Lunla Udomwech [a,b], Kulwadee Karnjana [a], Juntamanee Jewboonchu [a], Phisut Rattanathamma [b], Udomsak Narkkul [a], Jakkrit Juhong [a,b], Auemphon Mordmuang [a,*]

[a] School of Medicine, Walailak University, Nakhon Si Thammarat 80160, Thailand
[b] Walailak University Hospital, Walailak University, Nakhon Si Thammarat 80160, Thailand

ARTICLE INFO

Keywords:
Contact lens
Contact lens care behaviour
Bacterial microbiota
Ocular infections
Cuture-selected microbial community

ABSTRACT

Introduction: Contact lens (CL) wear has been reported to cause changes to the microbiome of the ocular surface. More insight into the alteration of this microenvironment can help to understand the pathogenesis of CL-related eye infections. Knowledge of the relationship between the CL wearer's behaviours and pathogens would help health care providers focus on each step of proper CL care. This study aims to determine the behaviours that might be associated with the community of bacteria on CL.

Methods: A cross-sectional design was performed using anonymous questionnaires to obtain demographic data and assess hygiene practices among volunteering wearers. The CLs used were collected to evaluate the prevalence of pathogenic bacteria associated with ocular infections by PCR and microbiota analysis.

Results: The bacterial microbiota study revealed a total of 19 genera and 26 isolated strains from 20 eligible CLs. *Enterobacter, Staphylococcus,* and *Achromobacter* were the main genus in this subject population. *Staphylococcus pasteuri* and *Achromobacter agilis* were the most common pathogens at 65% and 35%, respectively. *Enterobacter mori*, a nonpathogenic organism, was found to be the most predominant strain, accounting for 27.51% of the total bacterial constituents. The risk behaviour of CL wear that was significantly associated with *A. agilis* contamination was cleaning the CL case with tap water (P value = 0.04).

Conclusions: This is the first study focusing on the association between the culture selected microbial community on the CL surface and compehensive behavioural characteristics. Environmental contamination was the main source of microbes found on CL surfaces. An emphasis in patient education should be placed on careful handling during the CL care routine and managing the hygiene of the surroundings.

1. Introduction

The contact lens (CL) is a preferred choice for a large number of people for correcting refractive errors due to its ability to correct a wide range of refractive errors, ease of adaptation, and its practicality for an active lifestyle. Even in the absence of refractive errors, CLs are still a popular choice for cosmetics. More than 140 million people worldwide use CLs on a regular basis [1]; however, this may also come with complications, some of which are sight-threatening. Wearing a CL compromises the ocular surface in many ways, both from the lens itself and from unfavourable behaviours accompanied by CL wear. CL is a foreign body in the eye that can potentially foster pathogenic microorganisms.

Recently, over one million visits for keratitis and CL-related complications occurred each year in the USA [2], and one of the major important risk factors for microbial keratitis is the use of CLs [3]. Bacteria are the most common pathogen of CL-related eye infections [4, 5]. Both gram-negative and gram-positive bacteria, including *Staphylococcus aureus*, *S. epidermidis*, *Klebsiella* sp. *Acinetobacter* sp. and *Pseudomonas aeruginosa*, are recognized as the main bacterial pathogens of keratitis [6]. These organisms possessed the ability to attach and adhere to the CL surface [7, 8]. In particular, the biofilm formation of the bacteria promoted an interplay between specific properties of the CL surface and the organism [9].

Each type of CL material's unique chemical and physical properties, such as hydrophobicity, ionicity, and surface roughness, all contribute to the risk of infection. Interaction between the CL and the eye can lead to an altered state of the ocular surface [10]. The front and back surfaces demand more tears for covering, leading to dryness. The movement of

* Corresponding author.
E-mail address: auemphon.mo@wu.ac.th (A. Mordmuang).

https://doi.org/10.1016/j.heliyon.2022.e09038
Received 8 December 2021; Received in revised form 6 February 2022; Accepted 25 February 2022

the CL on the lubricant-deprived ocular surface puts the cornea at an even greater vulnerability to microabrasions [3]. Corneal oxygenation is reduced as the lens acts as a physical barrier, blocking normal tear-gas exchange. Prolonged wearing only worsens dryness and cellular hypoxia, making the cornea more susceptible to pathogens [11]. Environmental exposure to dust and water brings pathogenic bacteria into the already compromised corneal surface. Apart from the inevitable risk from the lens acting on the corneal surface, another modifiable but hazardous complication associated with CL wear is mainly due to human behaviour [12]. Mishandling of CLs can both compromise the ocular surface and bring microbial contamination into the CL care system and the eye. The majority of CL users fail to adhere to good CL care behaviours, an important risk factor for CL-related eye infection, putting 40.9 million CL wearers in the United States at risk for serious eye infections [7]. Poor CL hygiene accounts for 12–66% of CL-related eye infection cases [6]. Poor hygiene is an important problem that is perhaps underestimated. A surprising 50% of CL wearers are not compliant with simple hygiene, such as hand washing [13].

Regarding the many steps involved in cleaning, disinfecting, and storing reusable CLs, mistakes in different steps may affect bacterial contamination differently. The ability to identify the causative risk behaviour can greatly help eye care professionals and CL wearers to focus on the steps and important points to avoid CL-related eye infection. Normal conjunctival bacterial normal flora has traditionally been considered predominantly gram-positive, reflecting those found on the skin [14]. The scientific advancements of the second decade of this century has allowed researchers to overcome the limitations of traditional culture. Microbiome analysis allows a deeper understanding of the unique microbial community in each niche. The conjunctival core microbiota is composed of the genera *Corynebacterium*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Streptococcus*, *Millisia*, *Anaerococcus*, *Finegoldia*, *Simonsiella*, and *Veillonella* [15]. Human microbiomes help to regulate the homeostasis of human health and disease. The imbalance of the normal gut microbiota causes many noninfectious diseases, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and obesity [16, 17, 18]. From one study that investigated the ocular surface microbiota, a relationship between *Streptococcus* infection of the lens storage container and allergy symptoms related to CL wear was found [19].

Considering the available information, the ocular surface microbiome most likely plays a key role in maintaining ocular surface homeostasis, and its alteration could be linked to the development and progression of eye diseases. The purpose of this study was to clarify how CL care behaviours affect bacterial contamination by using microbiota analysis, which may help to reveal the causal relationship between microorganisms and behaviour. It would also allow health personnel to emphasize the importance of certain steps in CL care and would aid future studies in developing strategies to avoid such pitfalls. Furthermore, microbiome data reflect the state of the microenvironment, revealing changes in ocular microorganisms in persons wearing CLs that are a plausible cause for allergic symptoms [19]. A cross-sectional study was conducted to examine the relationship between risk behaviours and the main pathogens causing eye infections. The findings of this study provide more insights into the behaviour of Thai CL wearers that have been rarely studied.

2. Materials and methods

2.1. Study design and sample collection

A cross-sectional study was performed from November 2020 to March 2021. The study protocol was reviewed and approved by the Human Research Ethics Committee of Walailak University (WUEC-20-321-01) before the first volunteer was enrolled, in accordance with the tenets of the Declaration of Helsinki and with international restrictions on this study. A CL wearer was defined as a person who wore the CLs at least 5 days per week during the past month. The study population consisted of participants aged between 17 and 58 years who attended the ophthalmology clinic at Walailak University Hospital, Walailak University, and academic colleges in Nakhon Si Thammarat, Thailand. The subjects were recruited by a research assistant. Participants who were administered any topical medications of anti-allergic agents and/or antimicrobial agents within 2 months prior to initiation of the present study were excluded. The eligible lens wearers were advised to bring their CL with them for collection on the consultation day. Written informed consent was obtained from all subjects after the explanation of the study. Participants completed a validated, anonymous, self-administered questionnaire regarding personal demographic information, the use of CL behaviours, and CL hygienic practice. Furthermore, the CLs used were collected to study the characteristics of bacterial accumulation and community, which might be related to the personal hygiene of those individuals. The optometrist placed the CLs in the sterile CL storage case containing normal saline solution, along with the questionnaire, put these in the sample envelope and returned them to the laboratory on the same day.

2.2. Questionnaire

Each participant completed a 47-item, anonymous, standardized paper questionnaire, which provided demographic data and behaviour of CL wear. In total, 20 soft CLs were obtained from 20 CL wearers. The questionnaire was divided into 3 parts: personal information (5 items), CL-related behaviours (20 items), and assessment of hygienic practices (22 items). Demographic information was collected, including sex, age, educational level, underlying disease, and history of antimicrobial agent administration as exclusion criteria.

2.3. Laboratory sampling, bacterial isolation and DNA extraction

The CLs from the participants were obtained within 4 h of CL wear on the same day, received at the laboratory and aseptically transferred to a culture tube. The CLs were grown on brain heart infusion broth (BHI, HiMedia, India) at 37 °C for 48 h for recovery of bacterial cells. The culture was centrifuged at 10,000×g for 5 min at 4 °C, washed once with Tris-ethylenediaminetetraacetic acid (TE) buffer [10 mM Tris-HCl (pH 8.0), 1 mM ethylenediaminetetraacetic acid], and resuspended in 0.5 ml of TE buffer. An aliquot of 1 mL of all samples was centrifuged for 1 min at 15,000×g, and then the supernatant was discarded from the tubes for DNA extraction. Genomic DNA (gDNA) was extracted from the bacterial pellet in accordance with the manufacturer's protocol of the Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech, Ltd., New Taipei City, Taiwan). The pellet was resuspended in 200 μL of lysozyme and incubated at 37 °C for 30 min. The supernatant was removed, 20 μL of proteinase K was added to the tube, and the tube was incubated at 60 °C for 10 min. DNA was lysed and bound to the GD column. The gDNA was washed and eluted in a collection tube. The purified gDNA was collected into one microcentrifuge tube and centrifuged at 15,000×g for 30 s. The concentration of gDNA was determined spectrophotometrically in a Nanodrop instrument and kept at -20 °C until library construction.

2.4. Sequencing of 16S rRNA gene and microbiota analysis

For each sample, 10 ng of precipitate was used to amplify the V3 and V4 region of the 16S rRNA gene following the procedure developed by Illumina MiSeq System, [Primer:16S Amplicon PCR Forward Primer = 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTACGGGNGGCW GCAG, 16S Amplicon PCR Reverse Primer = 5'GTCTCGTGGGCTC GGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC and adaptor sequences: Forward overhang: 5′TCGTCGGCAGCGTCAGATGTGTATAA GAGACAG-(locus specific sequence) Reverse overhang: 5′ GTCTCG TGGGCTCGGAGATGTGTATAA GAGACAG-(locus specific sequence)]; this method also used molecular barcodes to enable multiplex sequencing as previously described [20]. Paired-end sequencing (2 × 150 base pairs [bp]) of these amplicons was performed on a desktop sequencer (MiSeq; Illumina, Inc., San Diego, CA, USA). 16S rRNA gene pipeline data acquisition incorporated phylogenetic and alignment-based approaches to

Table 1. Participant's demographic data and behaviors of contact lens wear.

Demographic data	N = 20 (%)
Sex	
Female	20 (100%)
Age	
≤18	3 (15%)
19-30	3 (15%)
31-40	8 (40%)
40-50	5 (25%)
>50	1 (5%)
Underlying disease	
Yes	4 (20%)
No	16 (80%)
Educational level	
High school	1 (5%)
Vocational/High vocational certificate	3 (15%)
Graduate	7 (35%)
Postgraduate	9 (45%)
Behavior of contact lens wear	
Lens wear experience	
1–5 years	8 (40%)
6–10 years	2 (10%)
more than 10 years	10 (50%)
Type of lenses	
Clear soft CL	11(55%)
Cosmetic CL	9 (45%)
Frequency of wear in a week	
1–3 day	0
4–6 day	4 (20%)
Everyday	16 (80%)
Duration of wear	
Less than 8 h	2 (10%)
More than 8 h	18 (90%)
Source of CL purchase	
Health care professionals	0
Non-health care professionals	20 (100%)
Source of disinfecting solution purchase	
Health care professionals	1 (5%)
Non-health care professionals	19 (95%)
Symptoms associated with CL wear	
Dryness	14 (70%)
Irritation	10 (50%)
Tearing	7 (35%)
Redness	6 (30%)
Itchiness	4 (20%)
Blurry vision	2 (10%)
Discharge	2 (10%)
History of eye infection associated with CL wear	
Yes	7 (35%)
Keratitis	3 (15%)
Conjunctivitis	2 (10%)
Blepharitis or hordeolum	2 (10%)
None	13 (65%)
Sleep with CL in	
Yes	9 (45%)
No	11 (55%)
Sharing CL with others	
Yes	0
No	20 (100%)
Exceed the recommended planned replacement	
Yes	15 (75%)
No	5 (25%)
Using expired CL solutions (opened for more than 3 months)	
Yes	7 (35%)
No	13 (65%)
Exposure to water during CL wear	
Yes	19 (95%)
No	1 (5%)
The use of eye drops in conjunction with CL	
Yes	13 (65%)
No	7 (35%)
First or second-hand smoker	
Yes	1 (5%)
No	19 (95%)
Time spent work with terminal screen per day	
Less than 12 h	9 (45%)
More than 12 h	11 (55%)
Exposure to air-conditioned environment	
Less than 12 h	12 (60%)
More than 12 h	8 (40%)
Use of makeup close to the eye	
Yes	15 (75%)
No	5 (25%)

(*continued on next page*)

Table 1 (*continued*)

maximize data resolution. Read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using FlASH v1.2.11 [21] with at least a 50-bp overlap and no more than 1-bp mismatch. Merged sequences were clustered into operational taxonomic units (OTUs) at a similarity cut-off value of 97% using the CD-HIT-OTU program. An expected error rate of 0.5 was applied for quality filtering. We mapped OTUs to the rDnaTools database to determine taxonomies [22]. QIIME was used to cluster the operational taxonomic units (OTUs), and constructed an OTU table from the output files generated in the previous two steps for downstream analyses of alpha diversity (observed OTUs, Chao 1 estimator, and Shannon diversity index; confirm species diversity), beta diversity (unweighted and weighted UniFrac; visualize the community diversity), and taxonomic trends (at the phylum and genus level).

2.5. Scanning electron microscopy (SEM)

Bacterial accumulation on the used CLs was examined by SEM on the representative CL surface as the contamination subjects. The lenses were cut into two pieces and fixed in 2.5% glutaraldehyde/0.1 M cacodylate buffer pH 7.4, at 4 °C overnight. The sample was resuspended in 0.1 M cacodylate buffer pH 7.4 at 4 °C and secondarily fixed with 1% osmium tetroxide (OsO_4) (OsO_4; Electron Microscopy Sciences, Hatfield, PA) in cacodylate buffer for 1 h at room temperature. All samples were dehydrated in a graded ethanol series of 20%, 40%, 60%, 80%, 90%, and finally two changes of absolute ethanol [23]. The lenses were subjected to electron microscopy at the Center for Scientific and Technological Equipment (CSE), Walailak University, according to the following protocol. Samples were dried immediately, mounted on aluminium stubs, and sputter-coated with gold in the vacuum chamber of a Cressington 108 Auto Sputter Coater (Cressington Scientific Instruments, UK). Visualization was performed under a scanning electron microscope (Merlin Compact, Zeiss, Germany).

2.6. Statistical analysis

All statistical analyses were performed using SPSS software (version 23.0; SPSS Inc., Chicago, IL). The data were analyzed for both descriptive and inferential statistics. Continuous variables were described using the mean and standard deviation. Independent categorical variables were

Table 2. CL wearers hygiene behaviors.

CL care behaviors	N = 20 (%)
Always check expiration date and integrity of packaging before use	
Yes	17 (85%)
No	3 (15%)
Check for the correct side (inside-outside) before use	
Yes	18 (90%)
No	2 (10%)
Start inserting and removing the lens from the same eye	
Yes	17 (85%)
No	3 (15%)
Continued using the lens that had been dropped	
Yes	10 (50%)
No	10 (50%)
Hand wash before putting in the CLs	
With water only	5 (25%)
With soap	14 (70%)
Not done	1 (5%)
Routine before putting in the CLs	
Rub the lenses	1 (5%)
Rinse the lenses	7 (35%)
Rub and rinse the lenses	8 (40%)
No management	4 (20%)
Hand wash before CLs removal	
With water only	4 (20%)
With soap	10 (50%)
Not done	6 (30%)
Routine after CLs removal	
Rub the lenses	1 (5%)
Rinse the lenses	3 (15%)
Rub and rinse the lenses	9 (45%)
None	7 (35%)
Products used to clean the CL	
CL cleaning solution	13 (65%)
NSS	5 (25%)
Tap water	2 (10%)
Soaking CLs in the cleaning solution for ≥6 h before reuse	
Yes	19 (95%)
No	1 (5%)
Fill CL case with fresh CL solution every day	
Yes	20 (100%)
No	0
Topping off the old cleaning solution	
Yes	7 (35%)
No	13 (65%)
Close the cap of the cleaning solution tightly after use	
Yes	15 (75%)
No	5 (25%)
Keep using the same bottle of cleaning solution for more than 3 months	
Yes	1 (5%)
No	19 (95%)
Close the CL case tightly after use	
Yes	19 (95%)
No	1 (5%)
Keep using the same case for more than 3 months	
Yes	4 (20%)
No	16 (80%)
Clean the CL case with	
Water only	9 (45%)
Water and soap	5 (25%)
With CL solution	6 (30%)
Not done	0
Clean the CL case daily	
Yes	9 (45%)
No	11 (55%)

described using frequencies and expressed as percentages. For the continuous variables, a t-test (two groups) was used to compare the groups. The chi-square test was used to examine bivariate associations between independent variables and pathogen-related eye infections. A P value <0.05 was considered statistically significant for the group comparison.

3. Results

3.1. Demographic data and behaviour of the CL wearers

A total of 20 CL wearers were enrolled and completed the questionnaires in this study. A summary of the participants' demographic data is shown in Table 1. All participants were female with a mean age of 35.2 years, ranging from 17-58 years. Forty-five percent of the participants had a postgraduate degree education. All subjects wore soft reusable CLs, with the majority wearing monthly disposable lenses and 80% of the participants wearing CLs every day. Although it is generally recommended not to wear CLs more than 8 h per day, this survey found that 90% of subjects wore CLs longer than the recommendation. All CLs were purchased from non-health care professionals. Most of the participants (95%) also bought CL care solutions from non-health care professionals. Moreover, data revealed that 35% had a history of eye infections, including keratitis, conjunctivitis, and blepharitis. The undesirable activities found in this study were skipping annual eye check-ups, wearing CLs in water (such as swimming, diving, and shower), exceeding the recommended planned replacement of CLs and storage cases, and applying eye makeup.

The CL wearers' hygiene behaviour is demonstrated in Table 2. Most of the subjects had good practices in CL care, such as checking the expiration of the CL product and solutions, checking the side of the lens, and washing hands with soap before putting in and taking off the CL. Impressively, most performed the correct routine by the drop-rub-rinse regimen after wearing the CLs and even before. Moreover, they mostly followed the correct routine regarding the use of the CL care solution and its storage case, such as always renewing the cleaning solution, keeping the bottle clean, and not using the same CL case for more than 3 months. However, half of the participants continued to use lenses that had been dropped, which might have been contaminated, and had an improper CL case care regimen by cleaning them with tap water only.

3.2. Culture selected bacterial community on CLs

The 20 CL samples were coded as CL-1 to CL-20. The bacteria that were cultured from the CLs were detected and identified by microbiota analysis at the phylum to species level by 16S rRNA amplicon sequencing data. A bacterial microbiota study revealed that a total of 19 genus and 26 isolated trains were obtained from all CLs. Among the genus, *Enterobacter, Staphylococcus, and Achromobacter* were the most abundant representing 27.51%, 26.18% and 17.41% of total population, respectively. The isolated strains and CL care behaviours of each subject are shown in Table 3. The bacterial constituents of each sample are illustrated in Figure 1. The overall abundance of bacteria showed that *Enterobacter mori, Staphylococcus pasteuri,* and *Achromobacter agilis* were the 3 most predominant species, representing 27.51%, 26.17%, and

Table 3. Sample collection and culture-selected microbial community.

Subject ID	Age, sex	Past-history of eye infection or symptoms	Behavior	Isolated species	% found on CL
CL-1	38, Female	Abscess	- Sleeping or napping in CLs - Exceed the recommended period of CL - Wearing in water - No hand-washing before taking out - Clean the CL case with tap water - Use CL case more than 3 months	*S. periodonticum* *G. adiacens*	98.91 1.09
CL-2	18, Female	Never	- Sleeping or napping in CLs - Exceed the recommended period of CL - Wearing in water - Without soap - Soaking CL with normal saline solution - Reuse old CL solution - Use CL case more than 3 months	*S. pasteuri* *S. periodonticum* *A. junii* *S. epidermidis* *B. albigilva*	87.24 9.03 3.67 0.04 0.01
CL-3	17, Female	Never	- Exceed the recommended period of CL and solution - Wearing in water - No hand-washing before taking out - Without soap - Soaking CL with normal saline solution - Reuse old CL solution	*E. mori* *K. intermedia* *A. agilis* *C. plantarum* *S. pasteuri*	39.10 35.49 24.83 0.53 0.04
CL-4	17, Female	Never	- Sleeping or napping in CLs - Exceed the recommended period of CL and solution - Wearing in water - Without soap - No rub and rinse CL - Reuse old CL solution	*S. pasteuri* *P. aeruginosa* *P. stutzeri* *A. agilis*	98.21 1.19 0.53 0.07
CL-5	46, Female	Keratitis	- Wearing in water - Clean the CL case with tap water - No clean CL case - Use CL case more than 3 months	*S. pasteuri* *B. albigilva*	99.99 0.01
CL-6	37, Female	Never	- Exceed the recommended period of CL - Shower while wearing CL - Soaking CL with normal saline solution	*A. agilis* *H. aquaticum* *B. albigilva* *S. periodonticum* *B. wiedmannii*	97.22 2.74 0.02 0.01 0.01
CL-7	33, Female	Never	- Exceed the recommended period of solution - Wearing in water - Soaking CL with normal saline solution	*E. mori* *B. albigilva*	99.99 0.01
CL-8	27, Female	Conjunctivitis	- Exceed the recommended period of solution - Shower while wearing CL - Without soap - Clean the CL case with tap water	*S. surfactantfaciens* *P. geniculata*	99.90 0.10
CL-9	31, Female	Never	- Exceed the recommended period of CL and solution - Wearing in water - Without soap - No rub and rinse CL before soaking - Reuse old CL solution - Clean the CL case with tap water	*S. pasteuri*	100.00
CL-10	49, Female	Never	- Exceed the recommended period of CL - Wearing in water - Clean the CL case with tap water	*P. geniculata* *A. agilis* *S. maltophilia* *E. mori* *S. pasteuri*	72.89 24.63 2.45 0.02 0.01
CL-11	48, Female	Conjunctivitis	- Sleeping or napping in CLs - Exceed the recommended period of CL - Wearing in water	*E. mori* *K. oryziphila* *A. soli* *K. intermedia* *S. surfactantfaciens*	75.03 16.09 8.79 0.06 0.03
CL-12	33, Female	Keratitis	- Sleeping or napping in CLs - Exceed the recommended period of CL - Wearing in water - No hand washing before putting on and taking out - Reuse old CL solution - Clean the CL case with tap water	*E. mori* *A. soli* *S. pasteuri* *R. kristinae*	89.93 8.71 1.24 0.13
CL-13	33, Female	Abscess	- Wearing in water - No rub and rinse CL before putting on - Soaking CL with tap water - Reuse old CL solution - Clean the CL case with tap water	*E. mori* *A. lactucae* *S. pasteuri* *A. calcoaceticus*	54.07 32.62 8.84 4.47
CL-14	58, Female	Never	- Sleeping or napping in CLs - Exceed the recommended period of CL - Wearing in water - No rub and rinse CL - Clean the CL case with tap water - Use CL case more than 3 months	*S. pasteuri* *P. aeruginosa*	50.20 49.80

(*continued on next page*)

Table 3 (*continued*)

Subject ID	Age, sex	Past-history of eye infection or symptoms	Behavior	Isolated species	% found on CL
CL-15	27, Female	Never	- Exceed the recommended period of CL - Wearing in water - No hand-washing before taking out - Reuse old CL solution	*A. agilis* *E. mori* *B. albigilva*	99.97 0.02 0.01
CL-16	47, Female	Never	- Sleeping or napping in CLs - Exceed the recommended period of CL - Wearing in water - No hand-washing before taking out - No rub and rinse CL before soaking - Soaking CL with normal saline solution	*B. wiedmannii* *D. tsuruhatensis* *S. pasteuri*	67.53 28.76 3.71
CL-17	26, Female	Never	- Exceed the recommended period of CL - Shower while wearing CL - No hand-washing before taking out - No rub and rinse CL	*A. agilis* *B. albigilva* *S. pasteuri* *E. mori*	99.95 0.03 0.01 0.01
CL-18	50, Female	Never	- Exceed the recommended period of CL and solution - Wearing in water - Clean the CL case with tap water	*S. pasteuri* *P. paludis* *R. planticola* *E. mori*	73.95 26.03 0.01 0.01
CL-19	39, Female	Never	- Sleeping or napping in CLs - Exceed the recommended period of CL and solution - Wearing in water - No hand-washing before taking out - Soaking CL with normal saline solution	*E. mori* *R. ornithinolytica* *R. planticola* *A. agilis* *K. oryziphila* *K. intermedia* *S. pasteuri*	92.00 3.47 2.05 1.46 0.89 0.10 0.02
CL-20	31, Female	Keratitis	- Sleeping or napping in CLs	*E. mori* *B. albigilva*	99.97 0.03

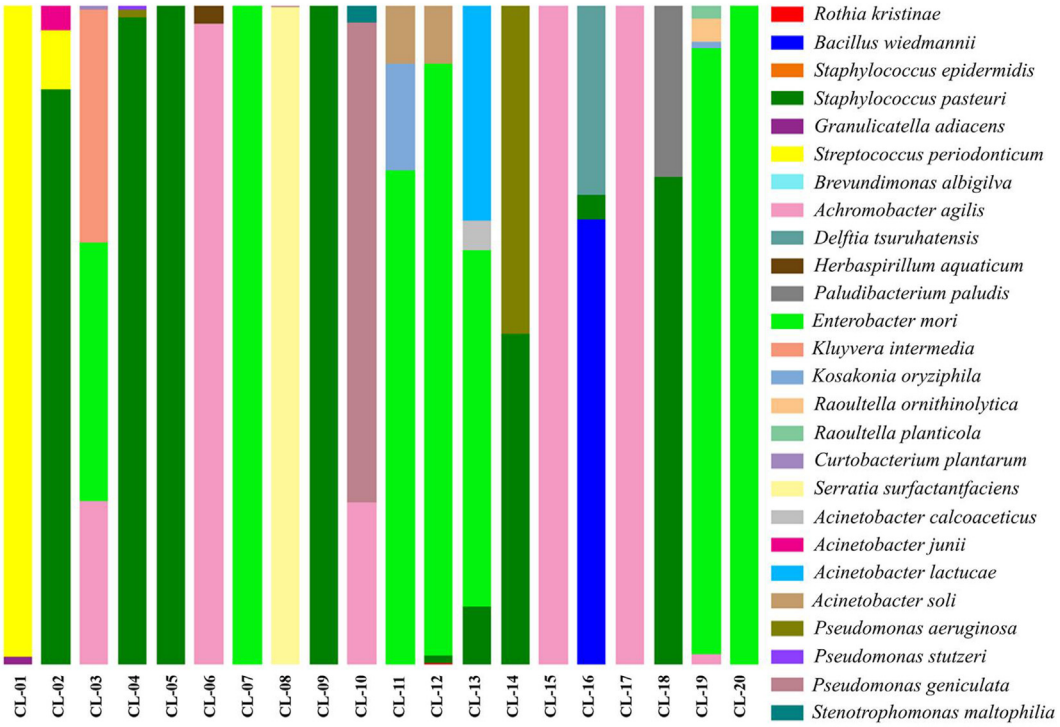

Figure 1. Stacked bar plots showing the percentage of bacterial populations as a taxonomic composition for each CL sample from the genus to species level. A total of 26 bacterial strains were identified in the subjects. *Enterobacter mori, Staphylococcus pasteuri,* and *Achromobacter agilis* were the 3 most predominant species, representing 27.51%, 26.17%, and 16.16% of the total population, respectively.

16.16% of the total population, respectively. Moreover, the bacterial isolates were justified as pathogens related to eye infections according to their contamination sources and background of causing the disease, as shown in Table 4. The main pathogens that were found in the present population were represented by 13 strains: *Serratia surfactantfaciens* (10%), *Staphylococcus epidermidis* (5%), *S. pasteuri* (65%), *Stenotrophomonas maltophilia* (5%), *Pseudomonas aeruginosa* (10%), *Delftia tsuruhatensis* (5%), *Acinetobacter calcoaceticus* (5%), *Granulicatella adiacens* (5%), *Raoultella planticola* (10%), *R. ornithinolytica* (5%), *A. agilis* (35%), *Pseudomonas stutzeri* (5%), and *Acinetobacter junii* (5%). Sources of

Table 4. Pathogens related eye infections.

Pathogens	% found in population (n = 20)	Source	References
S. surfactantfaciens	10	water and marine environments, contaminated soil, plants, animals, hospitalized patients	Grimont (2006) [24]; Su et al. (2016) [25]
S. epidermidis	5	human skin, upper respiratory tract	Du et al. (2021) [26]
S. pasteuri	65	drinking water, common skin flora, food products, air	Santoiemma et al. (2020) [27]
S. maltophilia	5	soil, sediment, wastewater, sputum	Ma et al. (2020) [28]; Al-Dhabi et al. (2021) [29]
P. aeruginosa	10	CLs, wet surfaces, chronic infection sites	Enzor et al. (2021) [30]; Riquelme et al. (2020) [31]
D. tsuruhatensis	5	soil, water, sludge, human microflora, CLs	Hotta et al. (2020) [19]
A. calcoaceticus	5	Soil, water	Roy et al. (2013) [32]
G. adiacens	5	human oral cavity, urogenital tract, gastrointestinal tract	Borroni (2002) [33]
R. planticola	10	vegetables, food, liquid soap	Vassallo et al. (2016) [34]
R. ornithinolytica	5	CLs, water, urine, wounds	Eguchi et al. (2017) [35];
A. agilis	35	rivers, ponds, residential water sources, soil, mud, some plants	Price et al. (2020) [36] Agbaji et al. (2020) [37] Vandamme et al. (2016) [38]
P. stutzeri	5	soil, water	Gilardi (1972) [39]; Lalucat et al. (2006) [40]
A. junii	5	water, soil, animals	Broniek et al. (2014) [41]

contamination are normally residential water, rivers, soil, mud, and some plants [19, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41], which might be related to the CL care routines and nonhygenic environments. For contamination by nonpathogenic species, 13 strains were found: *Brevundimonas albigilva*, E. *mori, Herbasprillum aquaticum, Kluyvera intermedia*, *Pseudomonas geniculata*, *Acinetobacter lactucae*, *Bacillus wiedmanni*, *Curtobacterium plantarum*, *Paludibacterium paludis, Kosakonia oryziphila*, *Rothia kristinae*, *Acinetobacter soli,* and Streptococcus periodonticum.

3.3. Risk behaviours of CL wear associated with eye infections

S. pasteuri and *A. agilis* were the most common organisms found from this studied population, at 65% and 35%, respectively. The two organisms were demonstrated to be representative of the main pathogens for evaluation of the association between CL wearers' behaviour and eye infection. Chi-square factors that showed the significance of the data are presented in Table 5. The two risk behaviours of cleaning the CL case with tap water and a prior history of eye infection were shown to be statistically significant in this study, with P values of 0.04 and 0.01, respectively.

3.4. Visualization of CLs

SEM was used to visualize the ultrastructure of bacterial accumulation on the CL surface. Figure 2A shows the surface of a CL that was taken from a control following the correct CL routine, such as hand washing with soap before applying and taking out the CL, not exceeding the recommended wearing period, and dropping-rubbing-rinsing the surface with CL care solution. This control was utilized as a reference for comparison with the CL from a subject with unfavourable behaviours (Figure 2B, C). The control CL shows a clear surface without bacterial attachment, whereas Figure 2B shows large amounts of bacterial adhesion. Additionally, the SEM photograph revealed typical biofilm morphologies and dense networks of cells arranged in multiple layers, forming microcolonies with the visible granular extracellular matrix of both gram-positive and gram-negative bacteria (Figure 2C).

4. Discussion

The ocular surface is a newly described niche with the unique characteristics of microbiota. Its consortium comprises a 'putative core' of 12 bacterial genera consisting of *Pseudomonas, Propionibacterium, Bradyrhizobium, Corynebacterium, Acinetobacter, Brevundimonas, Staphylococci, Aquabacterium, Sphingomonas, Streptococcus, Streptophyta,* and *Methylobacterium* [42, 43, 44]. The members of this putative core are the permanent residents of the ocular surface, despite occasional changes and the introduction of other bacteria. Recently, alteration of the ocular microbiome associated with CLs has been reported [45]. The microbial community in the presence of CL wear was found to be more variable than the community from the normal ocular surface, reflecting more of the skin flora, with higher abundances of *Methylobacterium*, *Lactobacillus*, *Acinetobacter*, and *Pseudomonas,* while *Haemophilus*, *Streptococcus*, *Staphylococcus*, and *Corynebacterium* showed lower abundances. Despite the close contact of the CL and the ocular surface, the microbiota of the CL surface was markedly different from those of the conjunctiva. The major microbes isolated from CL were previously reported, including coagulase-negative staphylococci (CNS), *Propionibacterium* sp., and *Corynebacterium* sp [46]. Another study of alterations in soft CL wearers also detected the constituents of *Streptococcus, Methylobacterium*, and *Acinetobacter* in the bacterial microbiome data [45].

This present study provided more insight into the nature of the bacterial microbiota on CLs. The 20 collected CLs contained 19 genera and 26 strains of bacteria. Additionally, 13 of these strains have been recognized as the causative pathogens of ocular infections or CL-related infections. The species with the highest predominance was *E. mori.* This bacteria is not a major pathogen causing disease in humans but rather in plants. However, it was mentioned in a case report as causing otitis externa in a 59-year patient in Austria [47]. Since bacteria are usually found in the rhizosphere and sometimes cause plant diseases, the detection of this pathogen at high levels indicates the contamination of soil and water [47].

The most ubiquitous gram-positive bacterium found in this study was *S. pasteuri,* a pathogenic bacterium with a coagulase-negative reaction that normally colonizes human skin or acts as a contaminant in water, food products, and unsanitary environments [23]. Contamination can occur by inappropriate handling of the CL during the process of wearing and taking off. Another *Staphylococcus* sp. found in this study was *S. epidermidis,* which was found in 5% of the studied population. This was in contrast to results from a previous study, which suggested that *Staphylococcus* sp. established on ocular surfaces in healthy adults represents as much as 73% of the bacterial community, especially *S. epidermidis* [48]. Aside from the skin flora discussed above, the gram-negative bacteria *A. agilis* showed a high abundance in the bacterial microbiota constituents in this study, which indicated that the contamination might be from pollutants in the surroundings [36, 47]. The source of *A. agilis* was previously study and the strain was obtained from soil as a rhizobacterial flora [37, 38]. The microbiota on the CLs reflected external

Table 5. Risk behaviors related to main pathogens causing eye infections.

Risk factor	N (%)	*Staphylococcus pasteuri*		P-value	*Achromobacter agilis*		P-value	*Enterobacter mori*		P-value
		Yes N (%)	No N (%)		Yes N (%)	No N (%)		Yes N (%)	No N (%)	
Sleeping or napping in CLs										
No	11 (55)	7 (53.85)	4 (57.14)	0.88	5 (71.43)	6 (46.15)	0.27	7 (63.64)	4 (44.44)	0.39
Yes	9 (45)	6 (46.15)	3 (42.86)		2 (28.57)	7 (53.85)		4 (36.36)	5 (55.56)	
Exceed the recommended period of CL										
No	5 (25)	2 (15.38)	3 (42.86)	0.17	0 (0)	5 (38.46)	0.058	3 (27.27)	2 (22.22)	0.75
Yes	15 (75)	11 (84.62)	4 (57.14)		7 (100)	8 (61.54)		8 (72.73)	7 (77.78)	
Exceed the recommended period of CL solution										
No	13 (65)	8 (61.54)	5 (71.43)	0.65	4 (57.14)	9 (69.23)	0.58	7 (63.64)	6 (66.67)	0.88
Yes	7 (35)	5 (38.46)	2 (28.57)		3 (42.86)	4 (30.77)		4 (36.36)	3 (33.33)	
Wearing in water										
No	4 (20)	1 (7.69)	3 (42.86)	0.06	2 (28.57)	2 (15.38)	0.48	2 (18.18)	2 (22.22)	0.82
Yes	16 (80)	12 (92.31)	4 (57.14)		5 (71.43)	11 (84.62)		9 (81.82)	7 (77.78)	
Wearing in shower										
No	3 (15)	1 (7.69)	2 (28.57)	0.21	1 (14.29)	2 (15.38)	0.94	3 (27.27)	0 (0)	0.08
Yes	17 (85)	12 (92.31)	5 (71.43)		6 (85.71)	11 (84.62)		8 (72.73)	9 (100)	
Hand washing										
No	6 (30)	4 (30.77)	2 (28.57)	0.91	3 (42.86)	3 (23.08)	0.35	2 (18.18)	5 (55.56)	0.49
Yes	14 (70)	9 (69.23)	5 (71.43)		4 (57.14)	10 (76.92)		9 (81.82)	4 (44.44)	
With soap										
No	10 (50)	7 (53.85)	3 (42.86)	0.63	5 (71.43)	5 (38.46)	0.16	5 (45.45)	5 (55.56)	0.65
Yes	10 (50)	6 (46.15)	4 (57.14)		2 (28.57)	8 (61.54)		6 (54.55)	4 (44.44)	
Rub and rinse CL										
No	7 (35)	6 (46.16)	1 (14.29)	0.15	3 (42.86)	4 (30.77)	0.58	2 (18.18)	5 (55.56)	0.08
Yes	13 (65)	7 (53.84)	6 (86.71)		4 (57.14)	9 (69.23)		9 (81.82)	4 (44.44)	
Reuse old CL solution										
No	13 (65)	7 (53.85)	6 (85.71)	0.15	4 (57.14)	9 (69.23)	0.58	7 (63.64)	6 (66.67)	0.88
Yes	7 (35)	6 (46.15)	1 (14.29)		3 (42.86)	4 (30.77)		4 (36.36)	3 (33.33)	
Soaking CL with normal saline solution										
No	15 (75)	10 (76.92)	5 (71.43)	0.78	4 (57.14)	11 (84.62)	0.17	8 (72.73)	7 (77.78)	0.79
Yes	5 (25)	3 (23.08)	2 (28.57)		3 (42.86)	2 (15.38)		3 (27.27)	2 (22.22)	
Clean the CL case with tap water										
No	11 (55)	6 (46.15)	5 (71.43)	0.27	6 (85.71)	5 (38.46)	0.04*	7 (63.64)	4 (44.44)	0.39
Yes	9 (45)	7 (53.85)	2 (28.57)		1 (14.29)	8 (61.54)		4 (36.36)	5 (55.56)	
History of eye infection										
No	13 (65)	10 (76.92)	3 (42.86)	0.12	7 (100)	6 (46.15)	0.01*	7 (63.64)	6 (66.67)	0.88
Yes	7 (35)	3 (23.08)	4 (57.14)		0 (0)	7 (53.85)		4 (36.36)	3 (33.33)	

Note: *A P value <0.05 was considered statistically significant from chi-square test.

contamination much more than that of the conjunctiva. The sources of the constituents on the lens surfaces were mostly from water, soil, the oral cavity, and the urogenital tract. This difference gave a clearer picture of the microenvironment of a CL wearer, with a population reflecting the environmental contaminants on the CL surface. Furthermore, the association of bacterial contamination with CL wearer behaviour was determined. The results of this study emphasize the danger of using tap water to clean CL cases. CL wearers who used tap water to clean the CL case carried a significantly higher risk of *A. agilis* contamination ($p = 0.04$). The CL case was recently acknowledged as the bulk of microbial contamination. The accumulation of bacteria and biofilm formation on the case surface was a commonly susceptible part of the CL care system more than the CLs themselves [49, 50]. This study provided insight into factors that may be significant in maintaining lens case hygiene and explored some of the issues previously proven in the in vitro study that tap-water use was associated with the contamination rate of gram-negative bacteria, particularly the strains *Pseudomonas* sp., *Stenotrophomonas maltophilia*, and *Achromobacter* sp [50]. Moreover, the bacterial pathogens that were reported in this study are commonly found on human skin, the oral cavity, and the urogenital tract, reflecting the non-compliance of CL wearers with hand washing. Seventy percent of the subjects routinely washed their hands before putting in CLs, and only half of them did so before taking off their CLs. The fact that most of the CL care process and CL case drying occurred in restrooms may increase urogenital tract pathogen contamination into the CL care system. In agreement with previous research, this study again highlights the negative effect of improper CL behaviour. The five most common improper CL care practices in Thai CL wearers were wearing CL for longer than recommended, not changing the CL solution, swimming with CLs, rinsing CLs with tap water, and not washing hands before handing the CLs [51]. Although our result showed that cleaning the CL case with tap water is statistically significant associated with *A. agilis* contamination. The non-significance difference of other behavior might be due to the low number of participants. Further studies of greater sample size would be necessary to confirm these findings.

The summation of this information suggests poor hygiene or overlooked pitfalls in CL handling. Most CL wearers received less-than-adequate to no education regarding CL handling at their time of purchase in conjunction with the surprisingly low proportion of CL wearers who seek their CLs and CL care solutions from a health care provider. Thus, no professional advice or patient evaluation for potential risks was ever provided. Appropriate behaviour remains a crucial point for eye care

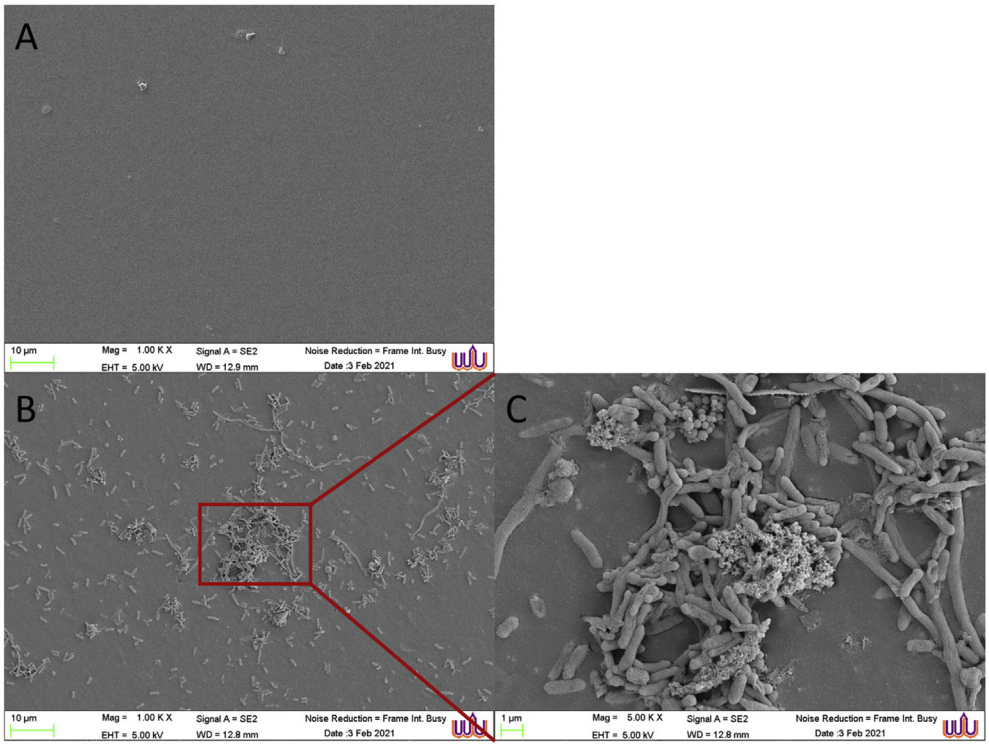

Figure 2. Scanning electron micrographs of bacterial accumulation on the CL surface. (A) A clear surface of a control CL with the correct care routine, such as hand washing with soap before applying and taking out the CL, not exceeding the recommended wearing period, and dropping-rubbing-rinsing the surface with CL care solution. (B,C) Markedly contaminated CL surface of a subject with unfavorable behaviours, at scale bars of 10 µm and 1 µm, respectively.

professionals to emphasize with their patients for a safe CL-corrected vision.

Statement of Ethics

The study protocol was reviewed and approved by the Human Research Ethics Committee of Walailak University (WUEC-20-321-01) before the first volunteer was enrolled, in accordance with the tenets of the Declaration of Helsinki and with international restrictions on this study.

Declarations

Author contribution statement

Lunla Udomwech: Conceived and designed the experiments; Wrote the paper.

Kulwadee Karnjana and Juntamanee Jewboonchu: Performed the experiments; Analysed and interpreted the data.

Phisut Rattanathamma, Udomsak Narkkul and Jakkrit Juhong: Analysed and interpreted the data.

Auemphon Mordmuang: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by Walailak University (Grant Number WU-IRG-63-081, 2020).

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors are grateful to thank the Research Center of Excellence in Innovation on Essential Oil and Research Institute for Health Science, and the Research Center in Tropical Pathobiology, Walailak University, for their kind support and laboratory facilities.

References

[1] C.H.L. Lim, F. Stapleton, J.S. Mehta, Review of contact lens-related complications, Eye Contact Lens 44 (Suppl 2) (2018) S1–s10.
[2] S.A. Collier, M.P. Gronostaj, A.K. MacGurn, et al., Estimated burden of keratitis–United States, 2010, MMWR Morb. Mortal. Wkly. Rep. 63 (45) (2014) 1027–1030.
[3] M. Eltis, Contact-lens-related microbial keratitis: case report and review, J. Optometr. 4 (4) (2011) 122–127.
[4] Y.T. Wu, M. Willcox, H. Zhu, F. Stapleton, Contact lens hygiene compliance and lens case contamination: a review, Contact Lens Anterior Eye 38 (5) (2015) 307–316.
[5] A.B. Zimmerman, A.D. Nixon, E.M. Rueff, Contact lens associated microbial keratitis: practical considerations for the optometrist, Clin. Optom. 8 (2016) 1–12.
[6] F. Stapleton, Contact lens-related corneal infection in Australia, Clin. Exp. Optom. 103 (4) (2020) 408–417.
[7] M.J. Giraldez, C.G. Resua, M. Lira, et al., Contact lens hydrophobicity and roughness effects on bacterial adhesion, Optom. Vis. Sci. 87 (6) (2010) E426–E431.
[8] M.J. Miller, D.G. Ahearn, Adherence of Pseudomonas aeruginosa to hydrophilic contact lenses and other substrata, J. Clin. Microbiol. 25 (8) (1987) 1392–1397.
[9] S. Dosler, M. Hacioglu, F.N. Yilmaz, O. Oyardi, Biofilm modelling on the contact lenses and comparison of the in vitro activities of multipurpose lens solutions and antibiotics, PeerJ 8 (2020) e9419.
[10] A. Mordmuang, L. Udomwech, K. Karnjana, Influence of contact lens materials and cleaning procedures on bacterial adhesion and biofilm formation, Clin. Ophthalmol. 15 (2021) 2391–2402.

[11] S.M. Fleiszig, D.J. Evans, Pathogenesis of contact lens-associated microbial keratitis, Optom. Vis. Sci. 87 (4) (2010) 225–232.
[12] T.H. Bui, H.D. Cavanagh, D.M. Robertson, Patient compliance during contact lens wear: perceptions, awareness, and behavior, Eye Contact Lens 36 (6) (2010) 334–339.
[13] D. Fonn, L. Jones, Hand hygiene is linked to microbial keratitis and corneal inflammatory events, Contact Lens Anterior Eye 42 (2) (2019) 132–135.
[14] R. Ratnumnoi, N. Keorochana, C. Sontisombat, Normal flora of conjunctiva and lid margin, as well as its antibiotic sensitivity, in patients undergoing cataract surgery at Phramongkutklao Hospital, Clin. Ophthalmol. 11 (2017) 237–241.
[15] F. Petrillo, D. Pignataro, M.A. Lavano, et al., Current evidence on the ocular surface microbiota and related diseases, Microorganisms 8 (7) (2020) 1033.
[16] J. Qin, Y. Li, Z. Cai, et al., A metagenome-wide association study of gut microbiota in type 2 diabetes, Nature 490 (7418) (2012) 55–60.
[17] T. Rinttilä, A. Lyra, L. Krogius-Kurikka, A. Palva, Real-time PCR analysis of enteric pathogens from fecal samples of irritable bowel syndrome subjects, Gut Pathog. 3 (1) (2011) 6.
[18] A. Swidsinski, J. Weber, V. Loening-Baucke, et al., Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease, J. Clin. Microbiol. 43 (7) (2005) 3380–3389.
[19] F. Hotta, H. Eguchi, H. Nakayama-Imaohji, et al., Microbiome analysis of contact lens care solutions and tear fluids of contact lens wearers: possible involvement of streptococcal antigens in allergic symptoms related to contact lens wear, Int. J. Mol. Med. 46 (4) (2020) 1367–1376.
[20] A.K. Bartram, M.D. Lynch, J.C. Stearns, et al., Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end illumina reads, Appl. Environ. Microbiol. 77 (11) (2011) 3846–3852.
[21] T. Magoč, S.L. Salzberg, FLASH: fast length adjustment of short reads to improve genome assemblies, Bioinformatics 27 (21) (2011) 2957–2963.
[22] P.D. Schloss, S.L. Westcott, T. Ryabin, et al., Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities, Appl. Environ. Microbiol. 75 (23) (2009) 7537–7541.
[23] L. Wiley, D.R. Bridge, L.A. Wiley, et al., Bacterial biofilm diversity in contact lens-related disease: emerging role of Achromobacter, Stenotrophomonas, and Delftia, Invest. Ophthalmol. Vis. Sci. 53 (7) (2012) 3896–3905.
[24] P.A. Grimont, F. Grimont, The genus serratia, Annu. Rev. Microbiol. 32 (1) (1978) 221–248.
[25] C. Su, Z. Xiang, Y. Liu, et al., Analysis of the genomic sequences and metabolites of Serratia surfactantfaciens sp. nov. YD25(T) that simultaneously produces prodigiosin and serrawettin W2, BMC Genom. 17 (1) (2016) 865.
[26] X. Du, J. Larsen, M. Li, et al., Staphylococcus epidermidis clones express Staphylococcus aureus-type wall teichoic acid to shift from a commensal to pathogen lifestyle, Nat. Microbiol. 6 (6) (2021) 757–768.
[27] P.P. Santoiemma, D.M. Kalainov, M.P. Mehta, M.K. Bolon, An unusual case of Staphylococcus pasteuri osteomyelitis, Infect. Dis. Rep. 12 (2) (2020) 8523.
[28] J. Ma, J. Feng, Y. Shan, et al., Characteristic antimicrobial resistance of clinically isolated Stenotrophomonas maltophilia CYZ via complete genome sequence, J. Glob Antimicrob. Resist. 23 (2020) 186–193.
[29] N.A. Al-Dhabi, G.A. Esmail, A.F. Alzeer, M.V. Arasu, Removal of nitrogen from wastewater of date processing industries using a Saudi Arabian mesophilic bacterium, Stenotrophomonas maltophilia Al-Dhabi-17 in sequencing batch reactor, Chemosphere 268 (2021) 128636.
[30] R. Enzor, E.M.R. Bowers, B. Perzia, et al., Comparison of clinical features and treatment outcomes of Pseudomonas aeruginosa keratitis in contact lens and non-contact lens wearers, Am. J. Ophthalmol. 227 (2021) 1–11.
[31] S.A. Riquelme, K. Liimatta, T. Wong Fok Lung, et al., Pseudomonas aeruginosa utilizes host-derived itaconate to redirect its metabolism to promote biofilm formation, Cell Metabol. 31 (6) (2020) 1091–1096.e6.
[32] R. Roy, P. Panigrahi, J. Malathi, et al., Endophthalmitis caused by Acinetobacter baumanni: a case series, Eye 27 (3) (2013) 450–452.
[33] D. Borroni, Granulicatella adiacens as an unusual cause of microbial keratitis: a metagenomic approach, Ocul. Immunol. Inflamm. (2021) 1–2.
[34] J. Vassallo, M. Vella, R. Cassar, P. Caruana, Four cases of Raoultella planticola conjunctivitis, Eye 30 (4) (2016) 632–634.
[35] H. Eguchi, F. Hotta, T. Kuwahara, et al., Acute keratoconjunctivitis due to contamination of contact lens care solution with histamine-producing Raoultella species: a case report, Medicine (Baltim.) 96 (50) (2017), e9310.
[36] E.P. Price, V. Soler Arango, T.J. Kidd, et al., Duplex real-time PCR assay for the simultaneous detection of Achromobacter xylosoxidans and Achromobacter spp, Microb. Genom. 6 (7) (2020).
[37] J.E. Agbaji, E.O. Nwaichi, G.O. Abu, Optimization of bioremediation-cocktail for application in the eco-recovery of crude oil polluted soil, AAS Open Res. 3 (2020) 7.
[38] P.A. Vandamme, C. Peeters, E. Inganäs, et al., Taxonomic dissection of Achromobacter denitrificans Coenye et al. 2003 and proposal of Achromobacter agilis sp. nov., nom. rev., Achromobacter pestifer sp. nov., nom. rev., Achromobacter kerstersii sp. nov. and Achromobacter deleyi sp. nov, Int. J. Syst. Evol. Microbiol. 66 (9) (2016) 3708–3717.
[39] G.L. Gilardi, Infrequently encountered Pseudomonas species causing infection in humans, Ann. Intern. Med. 77 (2) (1972) 211–215.
[40] J. Lalucat, A. Bennasar, R. Bosch, et al., Biology of Pseudomonas stutzeri, Microbiol. Mol. Biol. Rev. 70 (2) (2006) 510–547.
[41] G. Broniek, E. Langwińska-Wośko, J. Szaflik, M. Wróblewska, Acinetobacter junii as an aetiological agent of corneal ulcer, Infection 42 (6) (2014) 1051–1053.
[42] Q. Dong, J.M. Brulc, A. Iovieno, et al., Diversity of bacteria at healthy human conjunctiva, Invest. Ophthalmol. Vis. Sci. 52 (8) (2011) 5408–5413.
[43] Y. Huang, B. Yang, W. Li, Defining the normal core microbiome of conjunctival microbial communities, Clin. Microbiol. Infect. 22 (7) (2016) 643, e7-.e12.
[44] A. Omar, J.B. Wright, G. Schultz, et al., Microbial biofilms and chronic wounds, Microorganisms 5 (1) (2017).
[45] H. Shin, K. Price, L. Albert, et al., Changes in the eye microbiota associated with contact lens wearing, mBio 7 (2) (2016), e00198.
[46] M.D. Willcox, Characterization of the normal microbiota of the ocular surface, Exp. Eye Res. 117 (2013) 99–105.
[47] R. Hartl, H. Kerschner, R. Gattringer, et al., Whole-Genome analysis of a human Enterobacter mori isolate carrying a bla(IMI-2) carbapenemase in Austria, Microb. Drug Resist. 25 (1) (2019) 94–96.
[48] X. Wen, L. Miao, Y. Deng, et al., The influence of age and sex on ocular surface microbiota in healthy adults, Invest. Ophthalmol. Vis. Sci. 58 (14) (2017) 6030–6037.
[49] Y.T. Wu, H. Zhu, M. Willcox, F. Stapleton, The effectiveness of various cleaning regimens and current guidelines in contact lens case biofilm removal, Invest. Ophthalmol. Vis. Sci. 52 (8) (2011) 5287–5292.
[50] D. Tilia, P. Lazon de la Jara, H. Zhu, et al., The effect of compliance on contact lens case contamination, Optom. Vis. Sci. 91 (3) (2014) 262–271.
[51] C. Supiyaphun, P. Jongkhajornpong, Contact lens use patterns, behavior and knowledge among University Students in Thailand, Clin. Ophthalmol. 15 (2021) 1249–1258.